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Seroconversion in COVID-19 Infection and Comparison of Antibody Responses in Symptomatic Versus Asymptomatic Individuals

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Abstract

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus. Following infection, antibodies are formed against the spike (S) and nucleocapsid (N) proteins, which are the primary viral antigens of SARS-CoV-2. This study aims to determine the antibody response three weeks post-infection and its persistence. To study antibody responses in COVID-19-positive individuals and to compare the degree of antibody response in symptomatic and asymptomatic individuals. The persistence of the antibody response was also assessed. Adult patients (> 15 years of age) who were diagnosed as COVID-19-positive by RT-PCR, three weeks after swab positivity were tested for total antibody levels against COVID-19 antigens by electrochemiluminescence assay. Out of 226 individuals, 129 were symptomatic and 97 were asymptomatic. Among the 129 symptomatic individuals, 74 exhibited an antibody response, whereas in the asymptomatic individuals, only 10 exhibited an antibody response. The antibody response was found to be significant in symptomatic individuals compared to that in asymptomatic individuals ($p < 0.05$). All follow-up individuals were seropositive at the end of both 6 and 8 months. Antibodies against SARS-CoV-2 persist for 8 months following infection. Despite the waning of antibodies against the nucleocapsid antigen, there was no complete disappearance of antibodies.

Keywords: COVID-19, RT-PCR, Electrochemiluminescence Assay

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INTRODUCTION

Coronavirus disease (COVID-19) is an infectious disease caused by a newly discovered coronavirus.¹ Most people exposed to the COVID-19 virus will experience mild to moderate respiratory illness and recover with symptomatic management without requiring special treatment. The geriatric population and those with comorbidities, such as cardiac disease, diabetes, chronic respiratory illness, and malignancies, are more likely to develop serious illness with complications.² Following infection, antibodies are formed against the spike (S) and nucleocapsid (N) proteins, which are the primary viral antigens of SARS-CoV-2.³

After infection by SARS-CoV-2, different categories of antibodies circulate in the serum, such as immunoglobulin G (IgG), immunoglobulin M (IgM), and immunoglobulin A (IgA), mainly targeting two viral proteins, the S protein and nucleoprotein (NP).^{4,5} The N protein plays an important role in viral pathogenesis, replication, and RNA packaging. Antibodies to the N protein are frequently detected in COVID-19 patients.⁶

Seroconversion occurs between days 10 and 21 in most individuals infected with SARS-CoV-2.^{7,8} In cases involving a mild infection, it takes longer (four weeks or more) for seroconversion, and in a few cases, antibodies to SARS-CoV-2 are not detected during the follow-up period.⁹ Stronger antibody responses are related to a more severe disease status in patients with SARS-CoV-2 infection.¹⁰

While serological tests are now widely available, the correlates of immunity are not well established. Therefore, there remains a great need to measure antibody responses and determine seroconversion rates. While such serological assays are not well suited to detect acute infections, they help to identify antibody responses that correlate with protection from SARS-CoV-2.¹¹ There are several types of serological tests, including neutralization test, chemiluminescent immunoassay (CLIA), enzyme-linked immunosorbent assay (ELISA), and lateral flow assay (LFA). Serological assays could be employed to identify individuals who have been infected with SARS-CoV-2 and were asymptomatic or had mild symptoms, which include nasal congestion, rhinitis, and sore throat, thereby

providing a better understanding of how the virus is widely spread within a population.¹² This study sought to study the antibody response three weeks post-infection and its persistence.

MATERIALS AND METHODS

Place of study

This prospective cross-sectional study was conducted at the Institute of Microbiology, Madras Medical College, Chennai, Tamil Nadu, India.

Period of study

6 months (July 2020–December 2020)

Study design

Prospective cross-sectional study.

Ethics

Ethics Committee approval was obtained from the Institutional Ethics Committee of Madras Medical College, Chennai-3 (EC Reg. No. ECR/270/Inst./TN/2013/RR-16).

Statistical analysis

Statistical analysis was performed using SPSS 16. software The significance of the obtained results was determined using the chi-square test.

Adult patients (> 15 years of age) who were diagnosed as COVID-19-positive by RT-PCR three weeks after swab positivity were enrolled in the study. To evaluate the persistence of the antibody response, follow-up testing was performed at intervals of 3, 6, and 8 months.

Written consent was obtained after explaining the details of the study to all enrolled cases individually. Patients unwilling to provide consent and adult patients (> 15 years of age) who had a history of contact or symptoms suggestive of COVID-19 infection but were negative by PCR were excluded.

Under strict aseptic precautions, 4 mL of venous blood samples were collected and the serum was separated and stored at -20°C.

All collected samples were tested by electrochemiluminescence assay (Elecsys® kit) for in vitro qualitative determination of the total antibody titer to SARS-CoV-2 (nucleocapsid antigen), which is a test based on the principle of double-antigen sandwich assay.

Twenty microliters of serum samples were incubated with a mix of biotinylated and ruthenylated nucleocapsid (N) antigens. In the presence of the corresponding antibodies, double-antigen sandwich immune complexes were formed (9 minutes).

Double-antigen sandwich immune complexes bind to the solid phase via the interaction of biotin and streptavidin after the addition of streptavidin-coated microparticles. After an incubation period of 9 min, the reagent mixture was transferred to the measuring cell, where the microparticles magnetically captured onto the substances were subsequently removed. By applying voltage, electrochemiluminescence was induced, and the measurement was performed using a photomultiplier. The signal

yield increased with the antibody titer. Based on the manufacturer’s instructions, the results were interpreted as reactive if the cut-off index was ≥ 1.0 , and non-reactive if the cut-off index was ≥ 1.0 .

RESULTS

During the study period, 226 adult patients (> 15 years of age), 131 of whom were male and 95 female, diagnosed as COVID-19 positive by a positive RT-PCR test, were included in the study. Blood samples were collected three weeks after swab positivity and were tested for total antibodies against COVID-19. Out of the 226 samples, 84 were reactive and 142 were non-reactive.

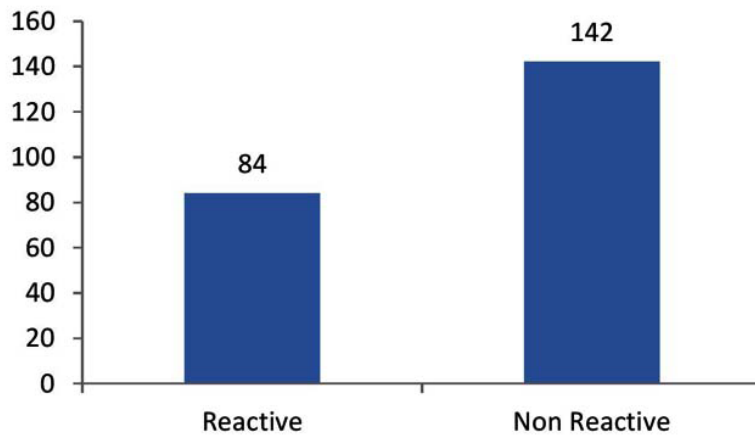


Figure 1. Antibody response(n=226)

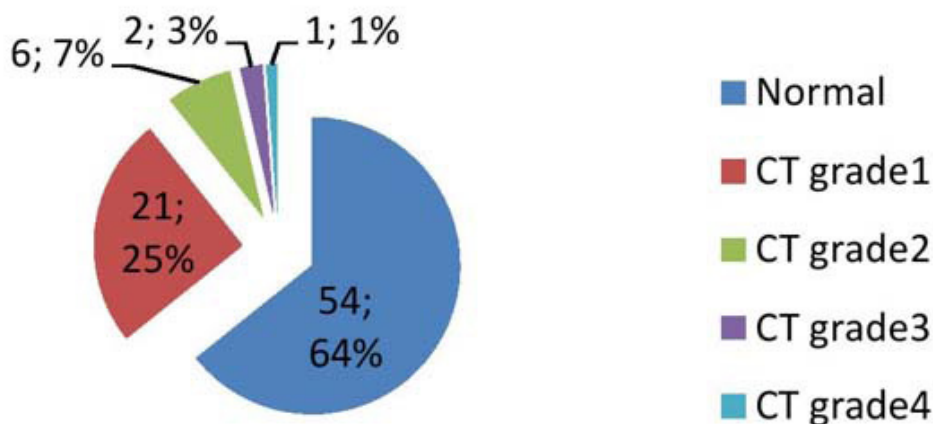


Figure 2. Correlation of CT chest changes with Antibody response(n=84 (reactive))

Table 1. Antibody response in symptomatic vs asymptomatic

Category	Symptomatic (n=129)	Asymptomatic (n=97)
Reactive	74	10
Non Reactive	55	87
P value	0.045 (P<.05) significant	

Approximately 74 (57%) of the 129 symptomatic individuals and 10 (10%) of the asymptomatic individuals exhibited positive antibody responses. Applying the chi-square test, the antibody response was found to be significant in symptomatic individuals compared to that in asymptomatic individuals ($p < 0.05$).

Among the reactive individuals, only 30 (36%) individuals came for the follow-up test after 3 months. The majority of them were under the category of grade 1 by CT chest examination (6%). Subsequently, at 6 and 8 months, 3 and 7 individuals, respectively, were lost to follow-up.

Out of 84 reactive individuals, 23 (27%) underwent the complete follow-up. Although some were lost to follow-up, individuals who reported for the entire period were seropositive at the end of both 6 and 8 months. The seroconversion rate among various grades of CT changes was not statistically significant.

DISCUSSION

The first human case of COVID-19 was first reported by officials in Wuhan City, China, in December 2019, and it was found to be caused by a novel coronavirus causing COVID-19, subsequently named SARS-CoV-2.¹¹ On 7 March 2020, a 45-year-old man who had returned from Oman with a history of fever, cough, and breathing difficulty was the first to test positive in the Indian state of Tamil Nadu. Following this case, there has been continuous reporting of SARS-CoV-2 positivity. To understand protective immunity, a serological assay based on the electrochemiluminescence assay principle was used in this study. The study included 226 adult patients (> 15 years of age) who were diagnosed as COVID-19-positive by RT-PCR and completed 3 weeks after swab positivity. Of these, 131 were males and 95 were

Table 2. Duration of Antibody response

Follow up month	CT grade1	CT grade2	CT grade3	CT grade4
3mts(n=30)	21	6	2	1
6mts(n=27)	18	6	2	1
8mts(n=23)	15	5	2	1
P value	0.999(not significant)			

females. RT-PCR is sensitive and specific for the diagnosis of COVID-19 early in the illness.¹³ An electrochemiluminescence assay method was used to assess the antibody response to nucleocapsid protein following infection in both symptomatic and asymptomatic individuals. Antibodies to the nucleocapsid protein are the most sensitive targets for the antibody response.¹⁴ There were 142 samples that were non-reactive and 84 that were reactive (Figure 1). In the study population, 129 (57%) individuals were symptomatic and 97 (43%) were asymptomatic. Approximately 74 of the 129 symptomatic individuals exhibited an antibody response compared to only 10 out of the 97 asymptomatic individuals. The antibody response was higher in the symptomatic individuals (57% vs. 10%; $p < 0.05$) than in those who were asymptomatic (Table 1). As the study involved only RT-PCR COVID-19 positives, recovery from infection in asymptomatic patients can be attributed to T-cell responses in the absence of humoral immunity. Asymptomatic positive subjects may benefit from some protective factors capable of limiting the spread of the virus within the body, or a more effective innate immune T cell-based response, as reported by Grifoni et al.,¹⁵ These factors could reduce the need for an adaptive response by the body, and consequently limit the production and persistence of antibodies.¹⁶ Tahmina et al.'s study involving 171 patients, of whom 108 were mildly symptomatic and 63 asymptomatic, also found a significant increase in the geometric mean titers of IgM, IgA, and IgG in mildly symptomatic patients compared to asymptomatic patients.^{17,18} In their study involving 214 patients, Jiang et al. observed seroconversion of immunoglobulin G in both asymptomatic and symptomatic patients, whereas immunoglobulin M seroconversion was less common in asymptomatic than in symptomatic patients (p -value < 0.001).¹⁹ With regard to the

correlation of the antibody response with CT chest findings (Figure 2), of the 84 seropositive individuals, 54 had normal CTs, and the majority of cases (21 patients, or 25%) had CT grade 1 changes. Six (7%) exhibited grade 2, two (3%) had CT grade 3, and one (1%) had grade 4 changes. Disease severity has been associated with the magnitude and duration of the antibody response.^{20,21} The persistence of antibody response was studied by performing follow-up testing at 3, 6, and 8 months. Out of 74 symptomatic, antibody-reactive individuals, 30 were followed up for persistence of the antibody response at 3, 6, and 8 months (Table 2). At 6 months, 3 individuals were lost to follow-up, and at 8 months, 7 were lost to follow-up. The reason for the loss to follow-up could be related to travel restrictions and the financial burden on individuals during the pandemic. All of the followed up individuals were seropositive at the end of 3, 6, and 8 months. The seroconversion rate and antibody duration for SARS-CoV-2 vary significantly across studies, depending on the serological tests used and the disease stage.^{22,23} Dan et al. analyzed cross-sectional data describing the dynamics of SARS-CoV-2 memory B cells, CD8+ T cells, and CD4+ T cells for more than 6 months after infection. The authors found a high degree of heterogeneity in the magnitude of adaptive immune responses, which persisted into the immune memory phase to the virus.^{24,25} Gudbjartsson et al. showed that the magnitudes and the durations of the antibody responses against SARS-CoV-2 are heterogeneous and vary widely between individuals.^{26,27} In our study follow-up at 6 months, there was a decline in the antibody titer compared to the early months following post-infection. Although there was a decline in the total antibody response, complete disappearance of antibodies was not observed throughout the entire follow-up period. Studies showed that the antibody titers of patients with mild SARS-CoV-2 infection declined more quickly than those reported for SARS-CoV patients, and waning immunity was confirmed 5 months after infection.²⁸ Choudhry et al. also reported waning of antibodies against nucleocapsid antigen, but not a complete disappearance after 16 weeks.²⁹ The antibody response predicts both mid-term and long-term clinical outcomes, and consequently

the use of monoclonal antibodies or boosting the antibody response with vaccination may be potential strategies to prevent COVID infection.³⁰ Further follow-up of the persistence of antibodies was not carried out as COVID-19 vaccination was started and there currently are no methods to differentiate vaccine-induced antibodies from antibodies resulting from natural infection.

CONCLUSION

Antibodies against SARS-CoV-2 persist for at least 8 months following infection. Despite the waning of antibody levels against the nucleocapsid antigen, there was no complete disappearance of antibodies. In addition to the antibody response, T-cell responses may play a role in limiting infection. As efforts are underway to vaccinate the majority of the adult population with two doses of vaccine, the findings of this study may be useful in making decisions regarding the timing of booster doses.

Limitations

Quantitative estimation of antibody responses and individual isotypes could not be carried out because of the non-availability of commercial kits and cost factors during the study period.

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None.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING

None.

DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT

Ethical clearance was obtained from the Institutional Ethics Committee (IEC), Madras Medical College, Chennai, India (EC Reg .No. ECR/270/Inst./TN/2013/RR-16).

INFORMED CONSENT

Written informed consent was obtained from the participants before enrolling in the study.

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